DEPARTMENT OF HEALTH & HUMAN SERVICES — PMS — Public Health Service

P930036 **Memorandum**

Date · SEP 29 1995

From Director, Office of Device Evaluation (HFZ-400)
Center for Devices and Radiological Health (CDRH)

Subject Premarket Approval of Ciba Corning Diagnostics Corporation's ACS™AFP - ACTION

To The Director, CDRH
ORA ___________

ISSUE. Publication of a notice announcing approval of the subject PMA.

FACTS. Tab A contains a FEDERAL REGISTER notice announcing:

(1) a premarket approval order for the above referenced medical device (Tab B); and

(2) the availability of a summary of safety and effectiveness data for the device (Tab C).

RECOMMENDATION. I recommend that the notice be signed and published.

for Susan Alpert, Ph.D., M.D.
Philip J. Phillips

Attachments
Tab A - Notice
Tab B - Order
Tab C - S & E Summary

DECISION

Approved ______ Disapproved ______ Date ______________________

Prepared by D.Moore, CDRH, HFZ-440, 9/27/95, 594-1293

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

[DOCKET NO. __________]

Ciba Corning Diagnostics Corporation; ACS™AFP

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing its approval of the application by Ciba Corning Diagnostics Corporation, Medfield, MA, for premarket approval, under section 515 of the Federal Food, Drug, and Cosmetic Act (the act), of ACS™AFP.

DATE: Petitions for administrative review by (insert date 30 days after date of publication in the FEDERAL REGISTER).

ADDRESS: Written requests for copies of the summary of safety and effectiveness data and petitions for administrative review, to the Dockets Management Branch (HFA-305), Food and Drug Administration, Rm. 1-23, 12420 Parklawn Drive, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT:

Peter E. Maxim, Ph.D.
Center for Devices and Radiological Health (HFZ-440)
Food and Drug Administration
9200 Corporate Blvd.

Rockville, MD 20850

301-594-1293.

SUPPLEMENTARY INFORMATION: On October 18, 1993, Ciba Corning Diagnostics Corporation, Medfield, MA 02052-1688, submitted to CDRH an application for premarket approval of ACS™AFP. The device is a two site chemiluminescence immunoassay and is indicated for the quantitative determination of alpha-fetoprotein (AFP) in human serum and in amniotic fluid from specimens obtained at 15 to 20 weeks gestation, as an aid in detecting open neural tube defects (NTDs), when used in conjunction with ultrasonography and amniography; and in human serum, as an aid in managing non-seminomatous testicular cancer, when used in conjunction with physical examination, histology/pathology, and other clinical evaluation procedures, using the Ciba Corning ACS:180® automated chemiluminescence system.

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Immunology Devices Panel, an FDA advisory panel, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

On September 29, 1995, CDRH approved the application by a letter to the applicant from the Director of the Office of Device Evaluation, CDRH.

A summary of the safety and effectiveness data on which CDRH based its approval is on file in the Dockets Management Branch (address above) and is available from that office upon written request. Requests should be identified with the name of the device and the docket number found in brackets in the heading of this document.

OPPORTUNITY FOR ADMINISTRATIVE REVIEW

Section 515(d)(3) of the act (21 U.S.C. 360e(d)(3)) authorizes any interested person to petition, under section 515(g) of the act (21 U.S.C. 360e(g)), for administrative review of CDRH's decision to approve this application. A petitioner may request either a formal hearing under part 12 (21 CFR part 12) of FDA's administrative practices and regulations or a review of the application and CDRH's action by an independent advisory committee of experts. A petition is to be in the form of a petition for reconsideration under 10.33(b) (21 CFR 10.33(b)). A petitioner shall identify the form of review requested (hearing or independent advisory committee) and shall submit with the petition supporting data and information showing that there is a genuine and substantial issue of material fact for resolution through administrative review. After reviewing the petition, FDA will decide whether to grant or deny the petition and will publish a notice of its decision in the FEDERAL REGISTER. If FDA grants the petition, the notice will state the issue to be reviewed, the form of the review to be used, the persons who may participate in the review, the time and place where the review will occur, and other details.

Petitioners may, at any time on or before (insert date 30 days after date of publication in the FEDERAL REGISTER), file with the Dockets Management Branch (address above) two copies of each petition and supporting data and information, identified with the name of the device and the docket number found in brackets in the heading of this document. Received petitions may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

This notice is issued under the Federal Food, Drug, and Cosmetic Act section 515(d), 520(h)(21 U.S.C. 360e(d), 360j(h)) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 5.10) and redelegated to the Director, Center for Devices and Radiological Health (21 CFR 5.53).

Dated: ______________________.

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
9200 Corporate Boulevard
Rockville MD 20856

Mr. William J. Pignato
Manager of Regulatory Affairs
Ciba Corning Diagnostics Corporation
63 North Street
Medfield, Massachusetts 02052-1688

SEP 29 1995

Re: P930036
Ciba Corning ACS™AFP Immunoassay
Filed: October 18, 1993
Amended: January 3, July 25, August 25, 1994; January 30, March 29, April 13 and 18, June 15, and September 29, 1995

Dear Mr. Pignato:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the Ciba Corning ACS™AFP Immunoassay. This device is indicated for the quantitative determination of alpha-fetoprotein (AFP) in human serum and in amniotic fluid from specimens obtained at 15 to 20 weeks gestation, as an aid in detecting open neural tube defects (NTDs), when used in conjunction with ultrasonography and amniography; and in human serum, as an aid in managing non-seminomatous testicular cancer, when used in conjunction with physical examination, histology/pathology, and other clinical evaluation procedures, using the Ciba Corning ACS:180® automated chemiluminescence system. We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

The sale, distribution and use of this device are restricted to prescription use in accordance with 21 CFR 801.109.

Expiration dating for this device has been established and approved at 12 months (one year) from date of manufacture when stored at 2-8°C. This is to advise you that the protocol you used to establish this expiration dating is considered an approved protocol for the purpose of extending the expiration dating as provided by 21 CFR 814.39(a)(8).

CDRH will publish a notice of its decision to approve your PMA in the FEDERAL REGISTER. The notice will state that a summary of the safety and effectiveness data upon which the approval is based is available to the public upon request. Within 30 days of publication of the notice of approval in the FEDERAL REGISTER, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the Federal Food, Drug, and Cosmetic Act (the act).

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

You are reminded that as soon as possible, and before commercial distribution of your device, that you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Peter E. Maxim, Ph.D. at (301) 594-1293.

Sincerely yours,

Philip J. Phillips

for Susan Alpert, Ph.D., M.D.
Director
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. GENERAL INFORMATION

Device Generic Name: Chemiluminometric Assay for Quantitation of Alpha-Fetoprotein (AFP) in Serum and Amniotic Fluid

Device Trade Name: **ACS™AFP** Assay

Applicant's Name and Address: Ciba Corning Diagnostics Corp.
63 North Street
Medfield, MA 02052-1688

Premarket Approval Application (PMA) Number: P930036

Panel Recommendations: Pursuant to section 515(c) (2) of the act as amended **by** the Safe Medical Devices Act of 1990, this PMA was not the subject of an FDA Immunology Devices Advisory Panel meeting because the information in the PMA substantially duplicates information previously reviewed by this panel.

Date of Approval of PMA: September 29, 1995

II. INDICATIONS FOR USE

The Ciba Corning ACS™AFP assay is indicated for the quantitative determination of alpha-fetoprotein (AFP) in human serum and in amniotic fluid from specimens obtained at 15 to 20 weeks gestation, as an aid in detecting open neural tube defects (NTDs), when used in conjunction with ultrasonography and amniography; and in human serum as an aid in managing non-seminomatous testicular cancer, when used in conjunction with physical examination, histology/pathology, and other clinical evaluation procedures using the Ciba Corning ACS:180® automated chemiluminescence system.

Background

AFP is a single chain glycoprotein with a molecular weight of approximately 70,000 daltons.[1] AFP was first described as a fetal protein by Bergstrand and Czar in 1956.[2] AFP and albumin share some physiological functions and considerable sequence homology.[3,4] Fetal AFP synthesis occurs in the liver, yolk sac, and gastrointestinal tract.[5] AFP produced by the fetus is secreted into fetal serum, reaches a peak at 13 weeks gestation, and then gradually declines during gestation. Shortly after birth, the newborn's AFP level reaches the low level observed in the normal adult. In adults, serum AFP concentrations remain low except during pregnancy, benign liver diseases (hepatitis, cirrhosis), hereditary diseases (ataxia telangiectasia, tyrosinemia) and certain malignancies such as primary hepatocellular carcinoma, and some germ cell tumors.[6-8]

Prenatal Testing

During pregnancy, maternal serum AFP (MSAFP) levels rise until the mid third trimester. Elevated or depressed AFP levels may indicate abnormal fetal pathology. Elevated MSAFP levels during the second trimester of pregnancy are associated with one of the most common types of birth defects, open neural tube defects (NTDs).[9-11] Transfer of AFP into the maternal circulation is accomplished primarily through diffusion across the placenta. If the fetus has an open NTD, AFP is thought to leak directly into the amniotic fluid and subsequently into the maternal circulation. A number of studies[12-16] have confirmed the utility of AFP testing to detect open fetal NTDs during the second trimester of pregnancy.

In evaluating the AFP test, maternal factors such as race, weight, age, diabetes, and family history must be considered when assessing the open NTD risk.[17,18] Final determination of open NTD depends on information provided by confirmatory testing since maternal conditions such as cirrhosis, hepatitis, and certain types of cancer, as well as other fetal malformations (ventral wall defects-omphalocele and gastroschisis[19] defective kidneys[20], and others), may also cause elevated MSAFP levels. Such testing includes amniography and ultrasonography. Depressed MSAFP levels have been reported in other conditions.[21-23]

Serum AFP testing is customarily done during the second trimester between gestation weeks 15 to 21. Normal median serum AFP levels rise from approximately 30 to 65 ng/mL during this time frame. Based on the U.K. Collaborative Study[12-14], the method of choice in reporting AFP levels is as a Multiple of the Median (MoM). Values from at least 100 patients are first determined for each gestational week to be reported. Median values are then established and subsequent individual results reported as multiples of this value. Values greater than 2.0 to 2.5 of the MoM, are cause for further investigation for the presence of fetal NTD.[17-20] Amniotic fluid AFP levels peak at about 12 weeks gestation after which they rapidly decline, tapering off at about week 22 of gestation and declining slowly for the duration of the pregnancy. When used in conjunction with other confirmatory tests, AFP measurement serves as a useful method in assessing risk for NTDs.

Cancer Management

As a tumor-associated antigen, AFP belongs to a class of molecules known as oncofetal proteins, among which are human chorionic gonadotropin (hCG) and carcinoembryonic antigen (CEA).[24] In 1964, Tatarinov's finding of AFP in the serum samples of hepatoma patients triggered extensive clinical studies.[25] Subsequently, Abelev and others not only confirmed and extended the clinical value of AFP in primary liver cancer, but also found AFP in testicular and ovarian cancers possessing elements of embryonic cancer.[26] Serum AFP measurement has now become a well accepted biochemical marker used as an aid in testicular cancer monitoring and in the detection of neural tube defects.[27-33,9-11] AFP testing has also proven useful in monitoring the progression of primary hepatocellular carcinoma (hepatoma) and may be of value in the effective management of patients with this malignancy.[34-39]

An important application of AFP testing in cancer management is for testicular cancer. The quantitative determination of AFP in human serum, when used in conjunction with physical examination, histological diagnosis, other established serum markers, and conventional evaluation procedures, has proven to be valuable in the management of patients with testicular tumors, especially nonseminomatous testicular cancer.[40] In nonseminomatous testicular cancer, AFP levels serve as both an aid in assessing the extent of disease and as a monitor of the effectiveness of therapeutic regimens and disease recurrence.

The collateral application of serial AFP monitoring, histological diagnosis, imaging technology, and other established markers as an integral strategy for the management of patients with nonseminomatous testicular cancer, has been well documented in the literature.[7,24,28-33] This combination of modalities has been proven to improve clinical staging, the determination of the presence of residual tumor after lymphadenectomy, monitoring the response to chemotherapy, and early detection of recurrence.

III. DEVICE DESCRIPTION

A. ACS Assay Procedure

The Ciba Corning ACS™AFP assay is a two-site solid phase sandwich immunoassay. The assay is designed for use on the Ciba Corning ACS:180®, a fully-automated, random-access immunoassay system. The ACS™AFP assay uses acridinium ester (AE) as the chemiluminescent tracer, magnetizable (paramagnetic) particles as the solid phase and an excess amount of two antibodies (rabbit polyclonal anti-AFP and mouse monoclonal anti-AFP) directed against epitopes on the AFP molecule.[41] The two reagents, when reacted with AFP from the sample, form a "sandwich" complex. The amount of AFP in the sample is directly related to the photons (as expressed in relative light units or RLU) detected by the ACS:180 system. Once the operator selects the ACS™AFP test, the system automatically conducts the following processes:

- Adds sample and ACS™AFP reagents to individual sample cuvettes.
- Conducts the reaction in a controlled temperature environment set at 37° C for 7.5 minutes.
- Magnetically separates, washes and aspirates fluid from the cuvettes to separate antibody-bound AFP from unbound tracer.
- Activates the AE chemiluminescent reaction by addition of hydrogen peroxide followed by addition of sodium hydroxide.

- Processes the RLU signal and converts it to a test result (reportable in concentration units of ng/mL AFP).

The AFP concentrations of controls and specimens are determined using two-point calibration and a stored Master Curve that covers the working range of the assay (0-1000 ng/mL). For specimens that lie outside this range, a manual dilution must be performed using a diluent provided as a separate reagent (Multi-Diluent 2). Amniotic fluids require a 1:80 dilution prior to testing to bring their higher values (μg/mL) within the working range of the ACS™AFP assay.

B. ACS™AFP Assay Reagents

The ACS™AFP assay includes the following reagents:

- AFP Lite Reagent is an affinity purified polyclonal rabbit anti-AFP antibody labeled with dimethylacridinium ester.[42] This material is used as the chemiluminescent tracer species in the reaction.

- AFP Solid Phase is a monoclonal mouse anti-AFP antibody covalently coupled to paramagnetic particles (PMP). These particles become magnetized when brought into a magnetic field, allowing separation of the added tracer into particle-bound and unbound species.

- ACS Calibrator D includes two vials of calibrator containing a low and high level of purified human AFP in a goat serum matrix. They are run periodically to adjust the system to a stored seven-point Master Curve which is prepared at the time of manufacture of new lots of reagents. Use of new lots of reagents requires the user to enter and store new calibration parameters into the system. This is achieved via transfer of data from a Master Curve Card with either the barcode reader or keyboard entry. Two point recalibration intervals may be maintained for up to 7 days.

C. Optional Reagents

- Multi-Diluent 2 consists of a goat serum matrix and is used to dilute patient sera and amniotic fluid with values above the standard range of the assay. Manual dilutions are required for the assay.

- Master Curve Material consists of seven ACS™AFP Master Curve standards ranging from 0 to greater than 1100 ng/mL. The ACS™AFP Master Curve standards are prepared with affinity purified human AFP (from umbilical cord serum) diluted in a goat serum matrix. These materials are made available (as optional reagents) to users of the ACS™AFP assay and can be used to assist in evaluating Quality Control requirements.

IV. ALTERNATIVE PRACTICES AND PROCEDURES

Prenatal Testing

Alternative and additional practices for aiding in the detection of fetal open NTDs include ultrasonography and amniography and the use of other immunological devices approved by FDA for the quantitative determination of AFP in human serum or amniotic fluid.

Cancer Management

Alternative and additional practices for aiding in the management of nonseminomatous testicular cancer include physical evaluation, histological diagnosis after orchiectomy, lymphadenectomy and lymph node biopsy, exploratory laparoscopy, lymphography, chest radiography, ultrasound, computed tomography, magnetic resonance imaging, and the use of other immunological devices for the quantitative determination of AFP and other markers in human serum for which there are approved PMAs.

V. MARKETING HISTORY

Since November 1992, Ciba Corning has made the ACS™AFP assay commercially available outside the United States. It is currently approved for use in Austria, Australia, Belgium, Brazil, Canada, Czechoslovakia, Denmark, France, Germany, Hong Kong, Israel, Italy, Japan, Malaysia, Mexico, The Netherlands, New Zealand, Norway, Poland, Singapore, Spain, Sweden, Switzerland, Taiwan, Turkey, United Kingdom, and Venezuela. In the time that it has been made available, the Ciba Corning ACS™AFP has never been withdrawn or recalled from the market for reasons of safety and effectiveness.

VI. ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Prenatal Testing

Patients undergoing evaluation for fetal NTD abnormalities should experience no adverse effects from this in vitro device when test results are evaluated in conjunction with further confirmatory testing and all available clinical information. However, in the event of a false positive result, even with follow-up testing, there is a chance that a healthy fetus may be incorrectly diagnosed as having an NTD. A false negative result could mean that an adverse condition affecting a fetus could be missed. Also, amniocentesis, which is part of the follow up procedure, presents a risk which the physician should discuss with the patient.

Cancer Management

Patients undergoing treatment for nonseminomatous testicular cancer should experience no adverse effects from this in vitro device, when test results are used as an aid in managing nonseminomatous testicular cancer in conjunction with other routine medical practices and procedures and all available clinical information.

A false positive result would indicate that a person may incorrectly be diagnosed as having testicular cancer. Conversely, a false negative result would indicate that there is no change in the patient's clinical status. However, additional or corrective diagnostic information would be obtained from results of conjunctive medical procedures.

VII. WARNINGS AND PRECAUTIONS

Use AFP results only as part of the overall clinical evaluation of a patient. Do not use AFP results as the only criterion for diagnosis.

The concentration of AFP in a given specimen, as determined by assays from different manufacturers, can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the AFP assay used. Values obtained with different AFP assay methods cannot be used interchangeably. Before changing assay methods the laboratory must do the following:

- For prenatal testing, the laboratory must establish a range of normal values for the new assay based on normal serum and amniotic fluid from pregnant women with confirmed gestational age.
- For cancer management, the laboratory must perform additional sequential testing to confirm baseline values for patients being serially monitored.

Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays.[43] Patients routinely exposed to animals or to animal serum products, can be prone to this interference and anomalous values can be observed. Additional information may be required for diagnosis. The Ciba Corning ACS™AFP assay uses antibodies from two animal species (rabbit and mouse) and routinely adds animal (goat) sera to its assay components to minimize the interfering response.

Prenatal Testing

It is important to collect maternal serum specimens for open NTD testing before amniocentesis. Significant quantities of AFP may pass into the maternal circulation during amniocentesis causing MSAFP levels to increase. Since the estimated half-life of AFP in serum is four to six days,[8,39] the MSAFP levels may be falsely elevated.

Treat amniotic fluid specimens contaminated with red blood cells with caution because fetal blood drawn with amniotic fluid may artificially elevate the AFP result. If contamination is suspected, then evaluate the amniotic fluid for the presence of fetal hemoglobin.

Elevated MSAFP levels may indicate open NTD, but are not used to diagnose the defect without additional testing. Incorrect estimation of gestational age can result in either under or overestimation of open NTD risk. Therefore, AFP testing requires accurate gestational dating for reliable risk assessment for open NTDs. Confirmatory procedures such as ultrasonography and amniography must be used in conjunction with MSAFP testing for accurate NTD risk assessment.

When using AFP in the evaluation of fetal defects, laboratories must establish their own median values for each gestational week. Absolute AFP values may vary for each lab depending on the demographics of their population including race and maternal weight.

Cancer Management

The ACS™AFP assay is not a screening test for cancer. AFP testing is a supplement to patient care when used as part of the overall management strategy for patients undergoing treatment for nonseminomatous testicular cancer, or for patients being monitored after therapy is complete.

Serum AFP can not be interpreted as absolute evidence of the presence of malignant disease. At time of presentation, patients with confirmed nonseminomatous testicular cancer may have serum AFP concentrations within the range observed in healthy individuals. Since elevated AFP levels are often found in patients with other malignant and non-malignant conditions, the physician should rule out all other conditions associated with elevated AFP levels prior to the use of the ACS™AFP values in nonseminomatous testicular cancer management. Conversely, low concentrations of AFP are not necessarily indicative of absence of disease, particularly post-surgery or after chemotherapy. Testicular tumors which are histologically categorized as pure seminoma do not synthesize AFP. The ACS™AFP assay, as a useful adjunct in cancer management, is intended for the evaluation of nonseminomatous testicular cancer, or mixed tumors with nonseminomatous elements, but not for pure seminoma. Additionally, several histologic subtypes of non-seminoma either do not synthesize AFP (choriocarcinoma) or do so unpredictably (teratoma). Therefore, AFP levels should be used concurrently along with other diagnostic and clinical patient information.

VIII. SUMMARY OF STUDIES

A. Preclinical Studies

Laboratory studies were performed at Ciba Corning to characterize the purity and specificity of the antigen and antibodies that are used to manufacture the ACS™AFP assay and to demonstrate the effectiveness of the ACS™AFP assay.

1. Characterization of the Antigen

AFP was isolated from human umbilical cord serum of newborns and purified to homogeneity by affinity chromatographic procedures. The antigen was characterized by the following techniques and compared to several reference sources of AFP.

Polyacrylamide gel electrophoresis (PAGE) demonstrated a predominant molecular weight species of approximately 70 kDa., for Ciba Corning AFP. This molecular weight is consistent with published values of AFP.[1] Additionally, comparative studies revealed no contamination with the major serum protein - albumin. Western blot analyses of Ciba Corning Reference AFP and other commercially available pure AFP preparations were equivalent, demonstrating similar staining of the monomeric form of AFP when utilizing a monoclonal antibody.

Amino acid composition analysis of Ciba Corning AFP demonstrated equivalent amounts of specific amino acid residues per mole AFP compared to literature values for both a purified AFP preparation and the theoretical amino acid composition based on the nucleotide sequence of human AFP mRNA.[1,4] Ouchterlony immunodiffusion studies using polyclonal anti-AFP antibodies demonstrated lines of identity with Ciba Corning AFP and pure AFP preparations obtained from commercial sources. These findings demonstrated the purity and identity of the antigens used in the standards and calibrators of the ACS™AFP assay.

2. Specificity of the Antibodies

   a. Monoclonal Antibody for the ACS™AFP Solid Phase

The antibody used for the ACS™AFP Solid Phase is a mouse monoclonal immunoglobulin. On SDS PAGE and agarose gel electrophoresis, following purification from ascites fluid, only 1 band was visible after staining. Both the molecular weight and electrophoretic mobility were consistent with that of gamma globulin. On SDS PAGE, under reducing conditions, two bands were visible with molecular weights consistent with isolated immunoglobulin heavy and light chain subunits. Subclass analysis demonstrated this antibody to be an IgG_1. Western blot analysis using a variety of highly purified AFP antigens showed only a single band of immunostaining for each preparation at a molecular weight of approximately 70 kDa, consistent with immunorecognition of monomeric AFP. Likewise, in the Western blot, this antibody exhibited no reactivity for either purified human serum albumin, or human plasma stripped of AFP, indicating a lack of cross reactivity by this antibody to albumin or other plasma proteins. Analysis of binding constants of the monoclonal antibody with purified AFP showed a K_a of 9×10^8 Liters/mole, indicative of a strong ligand binding interaction of AFP with this antibody.

These are accepted methods of characterization and have demonstrated the purity and specificity of the mouse monoclonal antibody used in the assay.

b. Affinity Purified Polyclonal Rabbit Antibody for the ACS™AFP Lite Reagent

Following affinity purification of this antibody on a resin composed of highly purified AFP, only a single band with a molecular weight of approximately 150 kDa was visible after protein staining on SDS-PAGE. Western blot analysis using Ciba Corning and other sources of AFP antigen showed a single band of immunostaining at a molecular weight of approximately 70 kDa, consistent with immuno-recognition of monomeric AFP. Additional faint bands, at the approximate molecular weights of AFP multimers were also visible consistent with literature reports of polyclonal antibody recognition of polymerized forms of AFP.[44] Also, there was no reactivity with this antibody in the Western blot to either purified human serum albumin, or human plasma stripped of AFP, indicating a lack of cross reactivity by this antibody for albumin or any other serum proteins except AFP. Ouchterlony immunodiffusion patterns showed reactivity of this antibody with several crude and purified AFP preparations from a number of sources. Determination of affinity binding constants by Scatchard plot using ^{125}I-AFP and affinity purified antibody resulted in a calculated K_a of 1.24×10^{11} Liters/mole, indicative of the very high affinity of this antibody for AFP.

These are accepted methods of characterization and have demonstrated the purity and specificity of the rabbit polyclonal antibody used in the ACS™AFP assay.

3. Performance Characteristics

a. Specificity

In addition to cross reactivity studies conducted on the individual antibodies against human serum albumin and other serum components, the monoclonal/polyclonal pair were evaluated for cross reactivity and interference in the ACS™AFP assay against a variety of endogenous serum components and chemotherapeutic drugs. High concentrations of hemoglobin (500 mg/dL), triglycerides (1000 mg/dL), bilirubin (conjugated and unconjugated-20 mg/dL) and serum proteins (9.5 gm/dL) did not interfere in the accurate determination of AFP.

Additional tests of both the monoclonal Solid Phase and affinity purified polyclonal Lite reagent revealed no cross reactivity with the following plasma proteins and other compounds which might be present during pregnancy: alpha-1-glycoprotein, alpha-1-antitrypsin, alpha globulin, ceruloplasmin, gamma globulin, chorionic gonadotrophin, placental lactogen, luteotropic hormone, transferrin, fetal hemoglobin, and pregnancy associated glycoprotein. Drugs such as acetaminophen and aspirin and vitamins commonly prescribed during pregnancy showed no cross reactive effects in the ACS™AFP assay.

Patient samples spiked with drugs commonly used in the treatment of cancer, especially testicular cancer, including bleomycin, vincristine, vinblastine, cisplatin, doxorubicin, cyclophosphamide, methotrexate, 5-fluorouracil, and mitomycin C had mean recoveries between 97 and 106.4 percent of the unspiked controls at all AFP concentrations tested. This demonstrated no interference with the AFP values determined.

b. Assay Range and Standardization

Seven AFP Master Curve standards are prepared with purified AFP in a goat serum matrix over the range of 0 ng/mL to greater than 1000 ng/mL. ACS™AFP is standardized by adjustment of Ciba Corning AFP to the internationally accepted primary AFP standard WHO IRP Lot # 72/225. One ng/mL AFP is equivalent to 0.83 IU/mL.

c. High Dose Hook Effect

Patient samples with high levels of AFP can cause a paradoxical decrease in RLU known as a high-dose hook effect or prozone. Due to this phenomenon, the possibility exists that extremely elevated levels of AFP could fall back within the working range of the curve resulting in a false low reading. In the ACS™AFP assay, samples with AFP levels as high as 1,000,000 ng/mL yielded results greater than 1000 ng/mL, the upper range cut off of the assay, and did not fall back within the working range of the assay.

d. Precision

i. Within-run and Run-to-run Precision

Within-run (intra-assay) and run-to-run (inter-assay) precision was evaluated at eight sites based on five runs at each site and three replicates in each run. Three controls and six patient pools with values across a wide range of AFP concentrations were tested. At five sites, three lots were run, for a total of 18 such test series (n = 270). Three different lots of ACS™AFP were used. Pooled within-run and run-to-run percent coefficients of variation (CV) are presented in TABLE 1. The CVs of 2.3 to 7.8 percent are acceptable for assays of this type.

TABLE 1. Pooled Within-run and Run-to-run Precision

Control	Mean AFP (ng/mL)	Pooled Within-run SD (ng/mL)	Pooled Within-run CV (%)	Pooled Run-to-run SD (ng/mL)	Pooled Run-to-run CV (%)
201A	34.8	1.3	3.6	2.0	5.8
201B	94.3	2.2	2.4	5.6	5.9
201C	213.9	5.7	2.7	12.1	5.7
Pool 1	18.9	0.7	3.9	1.5	7.8
Pool 2	50.3	1.3	2.7	2.7	5.4
Pool 3	91.0	2.4	2.6	5.4	6.0
Pool 4	155.8	3.6	2.3	9.1	5.9
Pool 5	652.3	16.6	2.5	44.3	6.8
Pool 6	819.4	21.0	2.6	60.0	7.3

ii. Site-to-site Precision

Site-to-site precision was evaluated at five trial sites. Three lots of ACS™AFP Solid Phase and Lite Reagent were tested at each site. Precision data from three controls and six patient pools at each site were determined for each lot and also pooled across the lots. Pooled site-to-site CVs ranged from 2.6 to 8.4 percent and are shown in TABLE 2. The CV results are acceptable for assays of this type.

TABLE 2. Site-to-Site Precision- 5 Sites, 3 Reagent Lots

	Lot # 01		Lot # 02		Lot # 04		Pooled by site	
Control	**Mean ng/mL**	**Site %CV**	**Mean ng/mL**	**Site %CV**	**Mean ng/mL**	**Site %CV**	**Mean ng/mL**	**%CV**
201A	35.3	4.9	33.8	3.6	35.3	5.5	34.8	4.8
201B	95.4	4.4	92.0	3.3	95.5	5.7	94.3	4.6
201C	216.5	3.2	209.3	4.0	216.0	4.8	213.9	4.0
Pool 1	19.1	8.0	18.2	5.7	19.4	5.2	18.9	6.4
Pool 2	50.9	4.2	49.4	2.6	50.7	5.4	50.3	4.3
Pool 3	92.1	5.3	89.5	3.4	91.3	5.0	91.0	4.6
Pool 4	157.9	3.2	153.4	4.2	156.0	6.8	155.8	4.9
Pool 5	658.2	4.4	649.1	5.2	649.6	6.9	652.3	5.4
Pool 6	823.7	4.1	821.5	5.4	813.0	8.4	819.4	6.0

iii. Lot-to-lot Precision

Lot-to-lot precision was evaluated using three lots of reagents at five trial sites. (Three additional sites used only one lot each.) Sites were analyzed individually and pooled lot variance was also calculated. Pooled lot-to-lot CVs ranged from 2.4 to 4.2 percent and are presented in TABLE 3. The CV results are acceptable for assays of this type.

TABLE 3. Pooled Lot to Lot Precision

Control	**Mean ng/mL**	**Lot %CV**
201A	34.8	3.5
201B	94.3	3.6
201C	213.9	2.4
Pool 1	18.9	4.2
Pool 2	50.3	2.8
Pool 3	91.0	3.0
Pool 4	155.8	3.0
Pool 5	652.3	3.1
Pool 6	819.4	3.4

These studies demonstrated acceptable precision and reproducibility of the ACS™AFP assay.

e. Analytical Sensitivity

The minimum detectable limit of the ACS™AFP assay was determined by diluting low patient specimens with zero (non-detectable AFP) standard. The sensitivity of the assay with a particular patient specimen was defined as the AFP concentration at which the photon count signal (RLU) was statistically different from the RLU signal generated at both zero AFP and the next lowest dilution of the patient sample. Statistical comparison was performed using the paired Student's t-test. The average sensitivity based on five patient profiles was 0.9 ng/mL.

When defined as two standard deviations above the zero standard, a sensitivity level of 0.3 ng/mL was calculated. Based on these two methods, the sensitivity of the ACS™AFP assay has been determined to be less than 1.0 ng/mL and is acceptable for an assay of this type.

f. Spiking Recovery

For 5 patient samples spiked with AFP concentrations ranging from 20 ng/mL to greater than 340 ng/mL, the mean recovery was 99 percent. These results demonstrated the ability of ACS™AFP to accurately quantitate AFP in different serum samples. The recovery results obtained are acceptable for assays of this type.

g. Dilution Recovery/Parallelism

Studies on dilution recovery and parallelism were split into three groups. The first group included samples with values less than 1000 ng/mL and were measurable within the dynamic range of the assay without requiring initial dilution. Patient sera with values above 1000 ng/mL (range 1200 to 350,000 ng/mL) were also assessed for their ability to dilute in a fashion parallel to the AFP standard curve in order to produce accurate results. Finally, amniotic fluid was tested for its ability to be diluted and retain linearity, as dilution of this type of sample is obligatory in order to bring the sample into the range of the ACS™AFP standard curve. All samples were diluted with Multi-Diluent 2.

Analysis of five patients in each group at five different dilutions resulted in mean recoveries of 105.2 percent, 102.2 percent and 101.2 percent, respectively.

These results confirmed that the ACS™AFP assay measured AFP in both serum and amniotic fluid in a

linear manner including samples with initial elevation well above the working range of the assay.

h. Carryover

AFP from elevated specimens should not significantly interfere with the accurate reading of subsequent samples being tested for AFP. This aspect of the ACS™AFP assay was tested to ensure minimal sample to sample contamination. The results of these tests demonstrated that a sample of 300,000 ng/mL caused an average increase of only 0.43 ng/mL in successive samples of a low concentration AFP sample (5.5 ng/mL). This carryover is within the sensitivity limits of the assay and indicated that the ACS 180 system was substantially free of sample to sample carryover effects.

i. Reagent Stability

The stability of the ACS™AFP assay components, AFP Lite Reagent and AFP Solid Phase, was assessed using three sequential manufactured lots of reagents. Evaluation of stability included testing of AFP standards, controls and calibrators at periodic intervals for up to 15 months. AFP levels (ng/mL) were compared to established specifications in order to assess reagent deterioration. Results of actual time-elapsed studies demonstrate no loss in performance after storage up to 18 months at 2-8° C and support dating of 12 months.

Studies of open bottle reagents kept onboard the ACS:180 demonstrate reagent stability of 40 hours, assuming ambient conditions of 30° C and 30 percent relative humidity.

Real-time stability studies on lyophilized standards and calibrators containing purified AFP demonstrated that they are stable for at least 15 months when stored at 2-8° C and support expiration dating of 8 months for calibrators and 15 months for standards. Following reconstitution, serum-based materials retained complete reactivity for up to 28 days when stored at 2-8° C. When placed in sample cups on the instrument, calibrator stability was maintained for up to 6 hours.

Real time analysis of Multi-Diluent 2 that was stored in liquid form at 2-8° C showed stability for greater than 12 months when evaluated in dilution recovery studies of a high concentration AFP sample. Expiration dating for Multi-Diluent 2 is 12 months.

j. Stored System Calibration

The ability of the ACS:180 system to maintain stored calibration of the ACS™AFP assay over 8 days was evaluated in the following manner:

- Calibration of the system was set on day zero using the two-point ACS Calibrator D to set the curve. Additionally, control samples were run throughout the entire curve range.

- On subsequent days of evaluation, additional Calibrator D was run as an unknown. This allowed patient and control data to be calculated with a daily system recalibration and compared to the results generated using stored calibrator values.

Equivalence of stored to daily calibration was confirmed by paired linear regression analysis of 527 patient samples with values across the working range of the assay. This study compared stored calibration over an 8 day period. The regression equation is:

Stored = 1.02 (Daily) - 0.833   r = 0.999

These results demonstrated the stability of the ACS™AFP assay calibration and validated a suggested calibration interval of 7 days.

k. Conclusion

The preclinical studies demonstrated that the device performance specifications for specificity, precision, analytical sensitivity, linearity, stability and other parameters are within acceptable limits.

B. Clinical Studies

1. Prenatal Testing

Clinical studies were performed with the ACS™AFP assay by four investigators at four centers. Described below are study objectives, study populations, distribution of serum and amniotic fluid AFP values for various gestational weeks and the development of representative median AFP values and multiple of the median charts.

a. Objectives

The main objective of these studies was to determine the safety and effectiveness of the ACS™AFP assay as an aid in detecting open neural tube defects (NTDs). This was achieved by testing the following parameters:

- Determination of the analytical precision of the ACS™AFP assay at each center, including assessment of lot to lot and site to site performance.
- Comparison at each site of AFP levels determined by ACS™AFP and an immunological device for AFP measurement for which there is an approved PMA.
- Measurement of maternal serum AFP levels by the ACS™AFP assay from gestational weeks 13 to 25; generation of pooled median AFP values from weeks 15 to 20 and calculation of selected multiple of the median (MoM) values.
- Measurement of amniotic fluid AFP levels by the ACS™AFP assay from gestational week 9 to 30; generation of pooled median values from weeks 15 to 20 and calculation of selected MoM values.

b. Clinical Sites

Four investigators participated in the clinical studies. One investigator utilized ACS:180 systems at two facilities for a total of five sites. The investigators and the respective institutions were:

- George Klee, M.D., Ph.D., Chair-Division of Metabolic and Hematologic Biochemistry, Department of Laboratory Medicine, Mayo Clinic Rochester, Rochester, MN

- Joseph Keffer, M.D., Director of Clinical Laboratories, Department of Pathology, Aston Clinic and Parkland Memorial Hospital (2 sites), Texas Southwestern Medical Center, Dallas, TX.

- A. Michael Spiekerman, Ph.D., Director of Clinical Chemistry, Department of Pathology, Scott and White Clinic, Temple, TX.

- Irene White, H.C.N., Head- Maternal Screening Unit, Department of Human Genetics, Royal Victoria Infirmary, Newcastle-Upon-Tyne, United Kingdom.

c. Study Populations

A total of 3482 maternal sera from 3431 women and 977 amniotic fluids from 974 women were used in the studies. The maternal sera specimens ranged in gestational week from 13 to 25 weeks. The amniotic fluid specimens ranged from 9 weeks to 30 weeks. As documented in the literature, factors taken into consideration included maternal weight, race, and diabetic status.[17,45-48]

d. Correlation of the ACS™AFP Assay to Comparative Methods

Correlations were run at three sites against two AFP devices for which there are approved PMAs. Maternal sera tested covered the range of 0 to greater than 450 ng/mL. Following dilution, amniotic fluids tested ranged from 2,000 to greater than 45,000 ng/mL (45μg/mL). Correlations for serum and amniotic fluid are presented in TABLE 4.

The correlations show that the ACS™AFP measured AFP in maternal sera and amniotic fluid in a manner comparable to other AFP devices for which there are approved PMAs.

TABLE 4. Correlation to Commercially available Devices for which there are approved PMAs.

Maternal Serum & Amniotic Fluid

Site	Comparative Assay	Specimen Type	N	Slope	Intercept AFP ng/mL	Correlation Coeff. (r)	S_{yx}
Scott & White	Method I	Maternal Sera Amniotic Fluid	504 103	0.94 0.91	4.6 2905.2	0.958 0.920	7.3 2446
Aston Parkland	Method II	Maternal Sera Amniotic Fluid	1575 500	0.92 0.87	6.2 865.7	0.985 0.906	5.2 2063
Mayo Clinic	Method II	Maternal Sera Amniotic Fluid	522 305	1.07 1.20	5.1 22.2	0.987 0.964	7.4 1985

e. Generation of Medians and Patient MoMs

Pooled maternal serum medians,using AFP values as measured by the ACS™AFP assay, were determined for gestational weeks 15 to 20. These maternal serum medians were comprised of 1,713 serum specimens obtained from three clinical trial sites. Regressed median AFP values generated from these sites are presented in TABLE 5. A site specific set of medians from 673 patients were used at the fourth site to generate MoMs. Regressed median values were determined using a weighted log linear regression.[17]

TABLE 5. AFP Medians for Pooled Maternal Sera (Aston, Mayo, Scott & White).

Gestational Week	# of Observations	Median ng/mL	Multiple of Median = 2.0	Multiple of Median = 2.5	Multiple of Median = 3.0
15	347	31.3	62.6	78.3	93.9
16	412	36.3	72.6	90.8	108.9
17	320	42	84	105	126
18	330	48.7	97.4	121.8	146.1
19	201	56.5	113	141.3	169.5
20	103	65.4	130.8	163.5	196.2

Amniotic fluid medians, using AFP values measured by the ACS™AFP assay, were determined for gestational weeks 15 to 20. These amniotic fluid medians were comprised of 714 amniotic fluid specimens from two clinical trial sites. The AFP regressed median values generated from the two clinical trial sites are presented in Table 6. One site had results that were not statistically poolable and another site did not perform amniotic fluid AFP. Values are reported as micrograms (μg)/mL.

The regressed medians and MoM values generated from the ACS™AFP data are similar to those found in published reports and to those generated from the ACS™AFP devices for which there are approved PMAs.

TABLE 6. AFP Medians for Amniotic Fluids (Aston, Mayo).

Gestational Week	# of Observations	Median μg/mL	Multiple of Median = 2.0	Multiple of Median = 2.5	Multiple of Median = 3.0
15	92	17.3	34.6	43.3	51.9
16	138	14.3	28.6	35.8	42.9
17	152	11.9	23.8	29.8	35.7
18	134	9.8	19.6	24.5	29.4
19	104	8.1	16.2	20.3	24.3
20	94	6.7	13.4	16.8	20.1

f. Clinical Sensitivity and Specificity

The Mayo Clinic assayed 236 maternal serum specimens and 87 amniotic fluid specimens at gestational weeks 15 to 20 with subsequently confirmed normal singleton pregnancies. None of these specimens had elevated AFP values using a cutoff of 2.5 multiples of the median. At this site, there were a total of 11 confirmed NTD birth outcomes from samples assayed at gestational weeks 15 to 20. Of these 11 specimens, 6 were maternal serum specimens and 5 were amniotic fluid specimens. At a cutoff of 2.5 multiples of the median, all the maternal serum and amniotic fluid specimens had elevated AFP values.

Of the 1429 maternal serum and 283 amniotic fluid samples assayed at Aston Clinic on gestational weeks 15 to 20, with normal singleton pregnancies confirmed at term, 26 maternal serum specimens and

4 amniotic fluid specimens had elevated AFP values using a 2.5 multiple of the median cutoff. There was one maternal serum specimen with a confirmed NTD birth outcome at gestational weeks 15 to 20 from the Aston Clinic. This maternal serum specimen was elevated using a cutoff of 2.5 multiple of the median.

The Royal Victoria Infirmary assayed 774 maternal serum specimens at gestational weeks 15 to 20 with normal singleton pregnancies confirmed at birth. Of these, six had an elevated AFP value using a cutoff of 2.5 multiples of the median. There were no amniotic fluid specimens assayed at this clinical trial site. There were 54 maternal serum specimens tested at Royal Victoria Infirmary on gestational weeks 15 to 20 with a confirmed NTD birth outcome. Elevated AFP values were recorded for 43 of these specimens at a cutoff of 2.5 multiples of the median.

Four hundred eighty maternal serum specimens, from gestational weeks 15 to 20, with normal singleton pregnancies confirmed at term, were assayed at the Scott & White Clinic and Hospital. Eleven of these specimens had elevated AFP levels, using a cutoff of 2.5 multiples of the median. No specimens assayed at this site had confirmed NTD birth outcomes.

Overall Clinical Sensitivity and Specificity

A total of 2,919 maternal serum specimens and 370 amniotic fluid specimens from confirmed normal singleton pregnancies were assayed at gestational weeks 15 to 20, at the four clinical trial sites. Using a cutoff of 2.5 multiples of the median, 43 of the maternal serum specimens and 4 of the amniotic fluid specimens had elevated AFP levels. Therefore, the clinical specificity of the ACS™AFP assay for maternal serum and amniotic fluid specimens was 98.5 (2876/2919) and 98.9 (366/370) percent, respectively. A total of 61 maternal serum specimens and 5 amniotic fluid specimens tested by the clinical trial sites at gestational weeks 15 to 20 were associated with confirmed NTD birth outcomes. Using a cutoff of 2.5 multiples of the median, 50 of the maternal serum specimens and 5 of the amniotic fluid specimens had elevated AFP levels. Therefore, the clinical sensitivity of the ACS™AFP assay for maternal serum and amniotic fluid specimens was 82 (50/61) and 100 (5/5) percent, respectively.

g. Conclusions

The performance parameters of the ACS™AFP assay closely resemble other valid AFP scientific studies presented in the literature.[12,13,17,49] and confirm the usefulness of the ACS™AFP assay as an aid in detecting open neural tube defects.

The Ciba Corning ACS™AFP assay is an acceptable method for the quantitative measurement of AFP in human serum and amniotic fluid at 15 to 20 weeks gestation to aid in the detection of open neural tube defects. The overall clinical specificity of the assay for maternal serum was determined to be 98.5 percent at a cutoff of 2.5 MoMs. The clinical specificity of the assay for amniotic fluid testing was found to be 98.9 percent for the same cutoff level.

The clinical sensitivity of the ACS™AFP assay for maternal serum was found to be 82.0 percent at the cutoff of 2.5 MoMs. The overall clinical sensitivity of the assay for amniotic fluid specimens was 100 percent at the same cutoff level. These performance parameters of the ACS™AFP assay are similar to those of other FDA approved AFP assays, and closely resemble other valid AFP scientific studies presented in the literature, which indicated its usefulness as an aid in detecting open neural tube defects.

2. Cancer Management

Clinical studies were performed with the ACS™AFP assay at four institutions. The protocol for the studies included study objectives, clinical sites, study population, distribution of AFP values within diagnostic categories, and comparison to AFP devices for which there are approved PMAs. Longitudinal patient studies (serial samples) demonstrated the clinical utility of the ACS™AFP assay.

a. Objectives

Retrospective studies were conducted at four clinical sites. The objectives of these studies were:

- to determine expected AFP ranges in apparently healthy populations, in patients with nonmalignant conditions, and in patients with nonseminomatous testicular cancer as well as other cancers.

- to compare AFP levels obtained with the ACS™AFP assay to those obtained with an AFP device for which there is an approved PMA.

- to assess the value of ACS™AFP levels in managing patients in whom nonseminomatous testicular cancer has already been diagnosed.

b. Clinical Sites

Four medical institutions and Ciba Corning Diagnostics Corp. participated in the clinical studies. The investigators and their respective institutions were:

- Herbert A. Fritsche, Ph.D., Chief of Clinical Chemistry, University of Texas, M.D. Anderson Cancer Center, Houston, Texas

- Morton K. Schwartz, Ph.D., Chairman, Department of Clinical Chemistry, Memorial Sloan-Kettering Cancer Center, New York, New York

- Laurence M. Demers, Ph.D., Professor of Pathology and Medicine, Department of Pathology, The M.S. Hershey Medical Center, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania

- George Klee, M.D., Chair-Division of Metabolic and Hematologic Biochemistry, Department of Laboratory Medicine, Mayo Clinic Rochester, Rochester, Minnesota

In addition to samples from the four clinical sites, longitudinal case study samples, obtained from Indiana University Medical School, and samples from individuals with cirrhosis, obtained from various hospitals, were assayed at Ciba Corning.

c. Study Population

The study included single specimens from 793 apparently healthy individuals and 348 patients with nonmalignant diseases. Specimens were also collected from 918 patients with malignant disease. The total number of specimens included in the clinical studies was 2,932. Not all of the specimens met inclusion criteria for each analysis.

d. Distribution of AFP Values

The percentage of 1858 individuals whose AFP levels fell between 0-8.0, 8.1-20.0, 20.1-500.0, 500.1-1000, and >1000 ng/mL are presented in Table 7 for all diagnostic groups.

Combining the four sites, the percentage of 793 healthy subjects with an ACS™AFP value less than 8.1 ng/mL was 98.4 percent.

Cancer patients from all sites represented primary and metastatic diseases. The types of cancers included in the study were testicular (nonseminomatous or seminomatous), primary liver, secondary liver, ovarian, gastrointestinal, genitourinary, pancreatic, and a miscellaneous category termed 'Other' for the remaining cancers. At a cutoff of 8.0 ng/mL, 49 percent of testicular nonseminomatous cancer patients, 10 percent of seminomatous patients, 64 percent of primary liver cancer patients, 15 percent of secondary liver cancer patients, and 16 percent of gastrointestinal cancer patients, 3 percent of genitourinary cancer patients, 6 percent of ovarian cancer patients, and 1 percent of pancreatic cancer patients had elevated AFP values. The percentage of other categories with elevated ACS™AFP values was 2 percent.

TABLE 7. **Distribution of AFP by Diagnostic Category**

Patient Category	No. of Samples	0.0 - 8.0 ng/mL.	8.1 - 20.0 ng/mL.	20.1 - 500 ng/mL.	500.1- 1000 ng/mL.	> 1000 ng/mL.
Apparently Healthy	793	780	12	1	0	0
Male	397	389	7	1	0	0
Female	396	391	5	0	0	0
Malignant Diseases	717	513	64	88	11	41
Testicular cancer						
seminoma	41	37	3	1	0	0
Nonseminoma	204	105	19	56	5	19
Liver cancer						
Primary	80	29	11	20	4	16
Secondary	93	79	8	5	0	1
Other cancer						
Gastroin-testinal	64	54	8	2	0	0
Genitouri-nary	40	37	3	0	0	0
Ovarian	78	73	5	0	0	0
Pancreatic	18	16	1	1	0	0
Other	99	83	6	3	2	5

Patient Category	No. of Samples	0.0 - 8.0 ng/mL.	8.1 - 20.0 ng/mL.	20.1 - 500 ng/mL.	500.1- 1000 ng/mL.	> 1000 ng/mL.
Benign Diseases	348	316	18	8	1	5
Cirrhosis	60	48	4	2	1	5
Hepatitis	64	51	8	5	0	0
Other	224	217	6	1	0	0

e. Correlation of the ACS™AFP Assay to Comparative Methods

Correlations were run against AFP devices for which there are approved PMAs using sera from normal populations as well as patients with benign conditions and various malignancies including nonseminomatous testicular cancer. Correlations within the working range of each comparative assay are shown in TABLE 8.

The correlation study demonstrated that the ACS™AFP test measures AFP levels in non-seminomatous cancer patients in a manner similar to other AFP kits for which there are approved PMA's.

TABLE 8. Correlations to Comparative AFP Methods

Site	Comparative Assay	No. of samples	Slope	Intercept	Correlation Coeff. (r)	S_{yx}
1	Method A	183	0.97	-1.0	0.99	8.8
2	Method A	281	0.89	0.4	1.00	3.3
3	Method B	477	1.10	-1.0	0.99	9.0
4	Method C	477	0.99	-0.6	0.99	2.4

f. Clinical Utility as Demonstrated by Serial Samples

To confirm the value of the ACS™AFP assay as an aid in the management of patients with nonseminomatous testicular cancer, serial (longitudinal) samples obtained from 131 patients were assayed for AFP using both the ACS™AFP assay and an assay for which there is an approved PMA. Trends in AFP values of the ACS™AFP and the comparative assay were examined. The ACS™AFP assay paralleled the comparative assays in 100 percent (131) of these case studies.

Ninety-seven of these serially monitored patients had nonseminomatous testicular cancer while 34 patients had a malignancy other than nonseminomatous testicular cancer. In 29 patients with nonseminomatous testicular cancer, clinical concordance of the assay could not be determined since the patients were disease-free throughout the study period. Clinical concordance was determined for the remaining 68 patients with nonseminomatous testicular cancer.

In 42 case studies in which the patient status was "no evidence of disease" or "complete remission" at the end of the study, 37 (88.1 percent) were concordant with the clinical history. In the 26 studies in which the patient status was "progressive" or "active disease" at the end of the study, 21 (80.8 percent) were concordant with the clinical history. The overall concordance for these 68 studies was 85.3 percent.

The serial monitoring study demonstrated the clinical utility of the ACS™AFP assay as an aid in the management of patients with non-seminomatous testicular cancer.

g. Conclusions

The Ciba Corning ACS™AFP assay demonstrated the ability to determine the concentration of AFP in human serum and to act as an aid in the management of nonseminomatous testicular cancer patients.

In clinical studies of apparently healthy individuals, patients with cancer and patients with a variety of non-malignant diseases, the ACS™AFP assay exhibited distribution results that parallel expected distributions for these patient types.

Method comparisons conducted at four external clinical sites showed acceptable correlation with the three AFP devices for which there are approved PMAs.

Patients monitored serially at four clinical trial sites were studied using the ACS™AFP assay and a comparative AFP device for which there is an approved PMA. The ACS™AFP assay was found to be highly concordant with the clinical histories of nonseminomatous testicular cancer patients. The ACS™AFP assay also showed an acceptable correlation to the comparative AFP device for which there is an approved PMA at each site.

IX. CONCLUSION

These data provide reasonable assurance that the ACS™AFP device is safe and effective as an aid in the detection of open neural tube defects and as an aid in the management of nonseminomatous testicular cancer patients.

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ACS®

AFP

Contents

Catalog Number	Contents	Number of Tests
672258	six vials of AFP Lite Reagent six vials of AFP Solid Phase one Master Curve card	300
or		
672259	one vial of AFP Lite Reagent one vial of AFP Solid Phase one Master Curve card	50

102110001 Rev. K, 10/95

Materials Required But Not Provided

Catalog Number	Description
672183	ACS Calibrator D (six vials each low and high)
or	
672173	ACS Calibrator D (two vials each low and high)
672261	ACS AFP Wash Reagent (100 mL/vial)
672013	ACS Septum Caps (100/package)

Optional Reagents and Supplies

Catalog Number	Description
672250	Multi-Diluent 2 (50 mL/vial)
672426	AFP Master Curve Material
976000	Tri-Level Ligand Control

Intended Use

For the quantitative determination of alpha-fetoprotein (AFP) in:

- human serum and in amniotic fluid from specimens obtained at 15 to 20 weeks gestation, as an aid in detecting open neural tube defects (NTDs), when used in conjunction with ultrasonography and amniography testing.
- human serum, as an aid in managing non-seminomatous testicular cancer, when used in conjunction with physical examination, histology/pathology, and other clinical evaluation procedures, using the Ciba Corning Automated Chemiluminescence Systems.

Warning: The concentration of AFP in a given specimen, as determined by assays from different manufacturers, can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the AFP assay used. Values obtained with different AFP assay methods cannot be used interchangeably. Before changing assay methods, the laboratory must do the following:

- For prenatal testing, the laboratory must establish a range of normal values for the new assay based on normal serum and amniotic fluid from pregnant women with a confirmed gestational age.
- For cancer management, the laboratory must perform additional testing to confirm baseline values for patients being serially monitored.

United States federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to by or on the order of a physician.

Use AFP results only as part of the overall clinical evaluation of a patient. Do not use AFP results as the only criterion for diagnosis. (Refer to Warnings, Summary and Explanation of the Test, and Limitations.)

Warnings

Elevated MSAFP levels may indicate open NTD, but are not used to diagnose the defect without additional testing. In addition, elevated MSAFP levels may indicate other forms of fetal distress or malformation, which include placental malformations, ventral wall defects, fetal kidney dysfunction, and fetal death. MSAFP levels may also be elevated in certain benign and malignant conditions not related to pregnancy. These conditions include hepatitis, cirrhosis, ataxia telangiectasia, primary hepatocellular carcinoma, and certain germ cell cancers. Furthermore, incorrect estimation of gestational age can result in either under or overestimation of open NTD risk. Therefore, AFP testing requires accurate gestational dating for reliable risk assessment for open NTDs. Confirmatory procedures such as ultrasonography, amniography, amniotic fluid acetylcholinesterase, and amniotic fluid AFP must be used in conjunction with MSAFP testing for accurate NTD risk assessment.

When using AFP in the evaluation of fetal defects, laboratories must establish their own median values for each gestational week. Absolute AFP values may vary for each lab depending on the demographics of their population including race and maternal weight.

Collect maternal serum specimens for NTD testing before amniocentesis. Refer to *Special Precautions* for detailed information.

The ACS™ AFP assay is not a screening test for cancer and must never be used as such. AFP testing is a safe and effective supplement to patient care when used as part of the overall management strategy for patients undergoing treatment for non-seminomatous testicular cancer, or for patients being monitored after therapy is complete.

Do not interpret serum AFP as absolute evidence of the presence of malignant disease. At time of presentation, patients with confirmed non-seminomatous testicular cancer may have serum AFP concentrations within the range observed in healthy individuals. Since elevated AFP levels are often found in patients with other malignant and non-malignant conditions, the physician should rule out all other conditions associated with elevated AFP levels prior to the use of the ACS AFP values in non-seminomatous testicular cancer management. Conversely, low concentrations of AFP are not necessarily indicative of absence of disease, particularly post-surgery or after chemotherapy. Testicular tumors that are histologically categorized as pure seminoma do not synthesize AFP. The ACS AFP assay, as a useful adjunct in cancer management, is intended for the evaluation of non-seminomatous testicular cancer, or mixed tumors with non-seminomatous elements, but not for pure seminoma. Additionally, several histologic subtypes of non-seminoma either do not synthesize AFP (choriocarcinoma) or do so unpredictably (teratoma). Therefore, AFP levels should be used concurrently along with other diagnostic and clinical patient information.

Summary and Explanation of the Test

AFP is a single chain glycoprotein with a molecular weight of approximately 70,000 daltons.[1] AFP was first described as a fetal protein by Bergstrand and Czar in 1956.[2] AFP and albumin share considerable sequence homology and some physiological functions.[3,4] Fetal AFP synthesis occurs in the liver, yolk sac, and gastrointestinal tract.[5] AFP produced by the fetus is secreted into fetal serum, reaches a peak at 13 weeks gestation, and then gradually declines during gestation. Shortly after birth, the newborn's AFP level reaches the normal adult level. In adults, serum AFP concentrations remain low except during pregnancy, benign liver diseases (hepatitis, cirrhosis), primary hepatocellular carcinoma, and certain germ cell tumors.

Prenatal Testing

During pregnancy, maternal serum AFP (MSAFP) levels rise through the third trimester. Elevated or depressed AFP levels may indicate fetal problems. Elevated MSAFP levels during the second trimester of pregnancy are often associated with one of the most common types of birth defects, open neural tube defects (NTDs).[6-8] A number of studies[9-12] have confirmed the utility of AFP testing to detect NTDs during the second trimester of pregnancy. In addition to AFP testing, maternal factors such as race, weight, age, diabetes, and family history must be considered when assessing the open NTD risk.[13-15] Final determination of open NTD depends on information provided by confirmatory testing since conditions other than open NTDs, such as cirrhosis, hepatitis, certain types of cancer, and other fetal malformations (ventral wall defects,[16] defective kidneys,[17] and others), may also cause elevated MSAFP levels.[18,19] Such testing includes amniotic fluid AFP (AFAFP), acetylcholinesterase, amniography, and ultrasonography. Depressed MSAFP levels have been reported in other conditions.

Cancer Management

Interest in AFP as a tumor marker originated with a report by Abelev in 1963.[20] Tatarinov provided the first evidence linking elevated serum AFP concentrations to primary cancer of the liver.[21] Since then, investigators have demonstrated elevated serum AFP levels in hepatocellular cancer,[22-23] malignant germ cell tumors of the ovary and testis,[24,25] and teratocarcinoma of the testis.[26] Although at a very low rate of incidence, increased circulating AFP concentrations may also occur in serum specimens from patients with gastrointestinal, pancreatic, and pulmonary cancers.[27]

The most important application of AFP testing in cancer management is for testicular cancer. Although not present in pure seminoma,[27] elevated serum AFP is closely associated with non-seminomatous testicular cancer.[28,29] The measurement of AFP in serum, in conjunction with serum hCG, is an established regimen for monitoring patients with non-seminomatous testicular cancer.[30-32] In addition, monitoring the rate of AFP clearance from serum after treatment is an indicator of the effectiveness of therapy.[33,34] Conversely, the growth rate of progressive cancer can be monitored by serially measuring serum AFP concentration over time.[35]

Serial serum AFP testing is a useful adjunctive test for managing non-seminomatous testicular cancer.

Note: ACS AFP physician brochures and patient brochures, explaining the use of AFP in prenatal testing to aid in the detection of fetal open NTD are available at no charge by calling the Ciba Corning Technical Assistance Center (toll free) at 1-800-255-2121.

Assay Principle

The Ciba Corning ACS AFP assay is a chemiluminescent sandwich assay, which uses constant amounts of two antibodies. The first antibody, or Lite Reagent, is an affinity purified polyclonal rabbit anti-AFP antibody labeled with acridinium ester. The second antibody, or Solid Phase, is a monoclonal mouse anti-AFP antibody covalently coupled to paramagnetic particles. The sample is incubated with both antibodies simultaneously for 7.5 minutes.

A direct relationship exists between the AFP concentration in the sample and the relative light units (RLUs) detected by the ACS:180 system.

Standardization

The ACS™ AFP assay is standardized against the World Health Organization International Reference Preparation 72/225[36,38] using highly purified AFP. The results are reported in ng/mL for serum AFP and in µg/mL for amniotic fluid AFP.

Specimen Collection and Handling

Serum and amniotic fluid are the recommended sample types for this assay. Tightly cap and refrigerate all specimens at 2–8°C if testing is not done immediately, or tightly cap and freeze all specimens at -20°C if testing is not done within 24 hours after collection. Freeze specimens only once and mix thoroughly after thawing. Avoid long-term storage in self-defrosting freezers.

Special Precautions

- Collect maternal serum specimens for open NTD testing before amniocentesis. Significant quantities of AFP may pass into the maternal circulation during amniocentesis causing MSAFP levels to increase. Since the estimated half-life of AFP in serum is four to six days,[40,41] the MSAFP levels may be falsely elevated.[42]
- Treat amniotic fluid specimens contaminated with red blood cells with caution because fetal blood drawn with amniotic fluid may artificially elevate the AFP result. If contamination is suspected, then evaluate the amniotic fluid for the presence of fetal hemoglobin.
- Centrifuge amniotic fluid specimens to clarify them before testing or freezing.
- Dilute all amniotic fluid specimens 1:80 using Multi-Diluent 2.

Assay Reagents

For In Vitro Diagnostic Use

Caution:

- Discard opened assay reagents that are at room temperature for a total of 40 hours; do not use these reagents to calibrate the system or assay samples.
- Do not use kit components beyond expiration date.
- Do not mix different lot numbers of reagents.

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Reagent	Volume	Ingredients	Storage	Stability
AFP Lite Reagent	2.5 mL/vial	purified polyclonal rabbit anti-AFP antibody (~0.4 μg/vial) labeled with acridinium ester in buffered saline, sodium azide (0.13%), and preservatives	2–8°C	until the expiration date on the vial label or cumulative 40 hours at room temperature
AFP Solid Phase	12.5 mL/vial	monoclonal mouse anti-AFP antibody (~0.8 mg/vial) covalently coupled to paramagnetic particles in buffered saline, sodium azide (<0.1%), and preservatives	2–8°C	until the expiration date on the vial label or cumulative 40 hours at room temperature
AFP Wash Reagent	100 mL/vial	NaCl (0.5 M) with detergent	18–25°C	until the expiration date on the vial label or cumulative 40 hours at room temperature

Warning: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush with a large volume of water to prevent the buildup of azides.

Biohazard: These reagents are intended for use in testing human bodily fluids. Components from biological sources were used in the manufacture of this product. It is recommended that appropriate biosafety precautions be followed to prevent the transmission of infectious agents.

Preparing the Assay Reagents

For best results, thoroughly mix the Solid Phase by inverting the vial before each use. Visually inspect the bottom of the vial to ensure that all the particles are dispersed and suspended. If foaming occurs, rim the top of the vial with applicator sticks to remove the foam.

Place a septum cap on the Lite Reagent and on the Solid Phase vials.

Prewarming or bringing the reagents to room temperature before use is not required.

Calibrating the Assay

For detailed information about entering calibration information, refer to Section 7, Calibration and Quality Control, in your ACS:180® Plus and ACS:180® Reference Manual.

Master Curve calibration

The ACS AFP assay requires a Master Curve calibration when you use a new lot number of Lite Reagent and Solid Phase. For each new lot of Lite Reagent and Solid Phase, use the Master Curve card to enter the Master Curve information at the System Management/Definitions/Master Curve screen. Six standards at levels in the range of 4, 40, 150, 300, 600, and 1100 ng/mL plus a 0 ng/mL standard are used in the preparation of each new Master Curve.

Two-point calibration interval

The suggested interval of the ACS AFP assay two-point calibration is every 7 days. This requires the use of low (~6 ng/mL) and high (~250 ng/mL) levels of Calibrator D, and is necessary to adjust the system to the Master Curve calibration.

Calibrator D contains highly purified AFP in a matrix of goat serum, sodium azide, and microbicides. Values are determined from a full seven-point Master Curve at the time of Calibrator D manufacture. *Reference values were initially determined for Master Curve standards as described in the section, Standardization.*

Also, perform a two-point calibration when you change lot numbers of Lite Reagent and Solid Phase or when your controls are repeatedly out of range.

Performing Quality Control

For detailed information about scheduling quality control materials, refer to Section 7, *Calibration and Quality Control*, in your *ACS:180® Plus and ACS:180® Reference Manual.* Enter quality control information at the System Management/Definitions/Controls screen.

To monitor system performance and chart trends, Ciba Corning recommends that a minimum of three levels of controls be run at least one time during an eight hour shift and when you perform a two-point calibration. If your established quality control program requires more frequent use of controls, then follow those procedures. Treat all quality control materials the same as patient samples.

For the ACS AFP assay, *Ciba Corning recommends using the Ciba Corning Tri-Level Ligand Control* or other controls for AFP that are cleared by the FDA. Refer to the Tri-Level Ligand Control product insert for the suggested control Value Range. If the quality control results do not fall within the suggested control Value Range, then do the following:

- review the ACS AFP product insert to ensure that you performed the assay according to the procedures recommended by Ciba Corning.
- check expiration dates to ensure that the materials you used are not expired.

If necessary, rerun the controls or contact Ciba Corning for more assistance.

Assay Procedure

For detailed information about operating the systems, refer to Section 6, *Operating the Systems*, in your *ACS:180® Plus and ACS:180® Reference Manual.*

Note: If automatic tray and cup assignment is on, use the printed worklist to help you load calibrators, controls, and patient samples into the correct tray and cup positions.

If automatic tray and cup assignment is off and your controls and patient samples are barcoded, you can load samples in any position.

1. Schedule the requested tests or profiles for each calibrator, control, or patient sample.
2. Prepare and load ACS Calibrator D.
   a. Reconstitute the low and high calibrators according to the preparation instructions in the ACS Calibrator D product insert.
   b. Dispense the low and high calibrators into a sample cup labeled with the appropriate barcode label.
   c. Load the sample cups in any position on the sample tray.

   Ensure that the low calibrator precedes the high calibrator on the sample tray.
3. Prepare and load controls.
   a. Prepare the controls according to the instructions in the control product insert.
   b. Dispense the controls into a labeled sample cup.
   c. Load the sample cup in the appropriate position on the sample tray.
4. Prepare the primary tubes or sample cups and load them on the sample tray.

   This assay requires 10 μL of sample for a single determination. This volume does not include the unusable volume in the sample cup or the additional volume required when performing duplicates or other tests on the same sample.
5. Fill a sample cup that is labeled with the appropriate barcode label with AFP Wash Reagent and load the sample cup on the sample tray. Each filled sample cup contains enough AFP Wash Reagent for approximately 20 tests.
6. Load the Lite Reagent and Solid Phase in adjacent positions on the reagent tray.
7. Press START. The ACS:180 system
   a. washes the sample probe with AFP Wash Reagent,
   b. dispenses 10 μL of sample into a cuvette,
   c. dispenses 50 μL of Lite Reagent and 250 μL of Solid Phase and incubates them for 7.5 minutes at 37°C,
   d. separates, aspirates, and washes the cuvette with reagent water,[43]
   e. dispenses 300 μL each of Reagent 1 and Reagent 2 to initiate the chemiluminescent reaction,
   f. prints results according to the print option you selected, which is described in Section 5, *Defining System Parameters*, in your *ACS:180® Plus and ACS:180® Reference Manual.*

Calculating Results

For detailed information about how the systems calculate results, refer to Section 2, *Understanding the Systems*, in your *ACS:180® Plus and ACS:180® Reference Manual.*

Procedural Notes

- Dispose of hazardous and biologically contaminated materials according to your institution's practices. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.
- Onboard dilution is not available for the AFP assay.
- Samples greater than 1000 ng/mL must be diluted and then repeated to obtain accurate results. Use Multi-Diluent 2 to dilute samples.
- Dilute all amniotic fluid samples 1:80 using Multi-Diluent 2.
- Use Multi-Diluent 2 to manually dilute serum samples with AFP values greater than 1000 ng/mL (1.0 μg/mL), and then load the diluted sample onto the sample tray, replacing the neat sample. Ensure that you mathematically correct the results for the dilution.
- To convert ng/mL (mass units) to IU/mL, use the factor 0.83 in the following equation:[44]

  1 ng/mL = 0.83 IU/mL

  Based on a molecular weight of 70,000 daltons 1 ng = 0.0143 nmoles

Limitations

Patient samples with high levels of AFP can cause a paradoxical decrease in the RLUs (high dose hook effect). In this assay, samples with AFP levels as high as 1,000,000 ng/mL read greater than 1000 ng/mL.

Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values can be observed. Additional information may be required for diagnosis. The Ciba Corning ACS AFP assay uses antibodies from two animal species and routinely adds animal sera to its assay components to minimize the interfering response.

Specimens that are . . .	have an insignificant effect on the assay up to . . .
hemolyzed	500 mg/dL of hemoglobin
lipemic	1000 mg/dL of triglycerides
icteric	20 mg/dL of bilirubin

Expected Values

AFP values in benign and malignant disease

The following data were obtained by testing a total of 1858 serum specimens.

Sample Category	No. of Samples	Distribution of AFP Values: 0–8.0 ng/mL	8.1–20.0 ng/mL	20.1–500.0 ng/mL	500.1–1000 ng/mL	> 1000 ng/mL
Apparently Healthy						
Subjects	793	780	12	1	0	0
males	397	389	7	1	0	0
females	396	391	5	0	0	0
Malignant Diseases	717	513	64	88	11	41
Testicular Cancer						
seminoma	41	37	3	1	0	0
non-seminoma	204	105	19	56	5	19
Liver Cancer						
primary	80	29	11	20	4	16
secondary	93	79	8	5	0	1
Other Cancer						
gastrointestinal	64	54	8	2	0	0
genitourinary	40	37	3	0	0	0
ovarian	78	73	5	0	0	0
pancreatic	18	16	1	1	0	0
other	99	83	6	3	2	5
Benign Diseases	348	316	18	8	1	5
cirrhosis	60	48	4	2	1	5
hepatitis	64	51	8	5	0	0
other	224	217	6	1	0	0

In this study, 98.4% of the apparently healthy subjects had AFP values less than 8.1 ng/mL.

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AFP values in maternal serum
The following data were obtained by testing a total of 1713 serum specimens at three sites. MSAFP values are reported in ng/mL.

Gestational Week	No. of Samples	Median* ng/mL	Multiples of Median (MoM, ng/mL) 2.0 MoM	2.5 MoM	3.0 MoM
15	347	31.3	62.6	78.3	93.9
16	412	36.3	72.6	90.8	108.9
17	320	42.0	84.0	105.0	126.0
18	330	48.7	97.4	121.8	146.1
19	201	56.5	113.0	141.3	169.5
20	103	65.4	130.8	163.5	196.2

*Medians are determined based on a weighted linear regression model.[14]

AFP values in amniotic fluid
The data below were obtained from 714 amniotic fluid samples at two sites.

AFP values are reported in µg/mL.

Gestational Week	No. of Observations	Median* µg/mL	Multiples of Median (MoM, µg/mL) 2.0 MoM	2.5 MoM	3.0 MoM
15	92	17.3	34.6	43.3	51.9
16	138	14.3	28.6	35.8	42.9
17	152	11.9	23.8	29.8	35.7
18	134	9.8	19.6	24.5	29.4
19	104	8.1	16.2	20.3	24.3
20	94	6.7	13.4	16.8	20.1

*Medians are determined based on a weighted linear regression model.[14]

Due to potential variability in AFP values attributable to differences in regional populations and assay methods, each laboratory should establish its own gestational age-specific median values. Various options for obtaining a reliable set of medians appropriate for your screened population have been described.[14] Once medians are available, it is customary to report AFP test results as a multiple of the median (MoM) to normalize for gestational age. Each laboratory must select a MoM screening cut-off that meets its needs.[8-11]

Performance Characteristics

Specificity
The potential interference of various endogenous proteins found in increased concentrations during pregnancy was tested by adding these proteins to serum pools containing AFP at three different concentrations. The AFP levels were then determined and compared to a serum control containing no added protein. Human proteins tested were: α_1-glycoprotein, α_1-antitrypsin, α-globulin, ceruloplasmin, chorionic gonadotropin, γ-globulin, placental lactogen, transferrin, luteotropic hormone, fetal hemoglobin, and pregnancy associated glycoprotein. There was no interference or cross-reactivity by any of these compounds. Common drugs such as aspirin and acetaminophen and vitamins commonly prescribed during pregnancy also demonstrated no interference in the measurement of AFP.

Interference by chemotherapeutic agents
The potential interference of chemotherapeutic agents was tested by adding these agents to serum pools containing AFP at three different concentrations. The AFP level was then determined and compared to a serum control that contained no chemotherapeutic agent.

Mean Recovery Substance	Amount Added	(Spike/control) x 100
Bleomycin	1300 µg/mL	101%
Cisplatin	1500 µg/mL	97%
Cyclophosphamide	330 µg/mL	101%
Doxorubicin	10 µg/mL	99%
5-fluorouracil	360 µg/mL	101%
Methotrexate	13 µg/mL	106%
Mitomycin-C	60 µg/mL	99%
Vinblastine	1200 µg/mL	100%
Vincristine	700 µg/mL	99%

Sensitivity
The minimum detectable limit of the ACS AFP assay was determined by diluting low patient specimens with zero standard. The sensitivity of the assay with a particular patient specimen was taken as that concentration which was statistically different from both the zero standard and the next lowest dilution of patient sample. The average sensitivity based on five patient profiles was 0.9 ng/mL.

Accuracy and recovery
For serum samples in the range of 0 to > 450 ng/mL, the correlations of the ACS AFP assay to four reference assays are described in the table:

Site	Reference AFP assay	No. of Samples	Slope	Intercept	Correlation Coefficient (r)
1	Method I	504	0.94	4.6	0.96
2	Method II	1575	0.92	6.2	0.99
3	Method III	183	0.97	−1.0	0.99
4	Method IV	477	1.1	1.0	0.99

For amniotic fluid samples in the range of 2 to 45 µg/mL, the correlations of the ACS AFP assay to three reference AFP assays are described in the table:

Site	Reference AFP assay	No. of Samples	Slope	Intercept	Correlation Coefficient (r)
1	Method I	103	0.91	2.91	0.92
2	Method II	500	0.87	0.86	0.91
3	Method III	305	1.20	0.02	0.96

Five serum samples with AFP concentrations ranging from 77 to 949 ng/mL were serially diluted up to five times in Multi-Diluent 2 and assessed for recovery and parallelism. The mean recovery was 105.2% with a range of 94 to 119.9%.

Five amniotic fluid samples with AFP concentrations ranging from 6.6 to 12.6 µg/mL were serially diluted up to five times following an initial dilution to bring the sample within the range of the ACS AFP assay. The mean recovery was 101.2% with a range of 94 to 113%. The first measurable dilution was assigned a value of 100%.

Precision
Three controls and six patient pools were tested at eight sites with five runs at each site and three replicates in each run. Five of the eight sites ran three lots of reagents, and three others ran only one lot of reagents, for a total of 18 such series (N = 270). Stored two-point calibration was used to determine AFP levels of the controls and patient pools.

Pooled within-run and run-to-run CVs are presented in the table:

Samples		Mean AFP (ng/mL)	Pooled Within-run % CV	Pooled Run-to-run % CV
Control	A	34.8	3.6	5.8
	B	94.3	2.4	5.9
	C	213.9	2.7	5.7
Pool	1	18.9	3.9	7.8
	2	50.3	2.7	5.4
	3	91.0	2.6	6.0
	4	155.8	2.3	5.9
	5	652.3	2.5	6.8
	6	819.4	2.6	7.3

Spiking
Known amounts of AFP ranging from 20 to 340 ng/mL were added to five patient samples with endogenous AFP levels between 30 and 50 ng/mL. Compared to expected values, the measured (recovered) levels of AFP averaged 99% with a range of 92 to 109%.

Carry-over
No significant carry-over was detected (less than 0.0002%) when a sample containing 300,000 ng/mL of AFP was assayed.

Troubleshooting the Assay
For detailed information about troubleshooting the ACS AFP assay on the ACS:180 systems, refer to Section 3, *Evaluating Assay and System Performance*, in your *ACS:180® Plus and ACS:180® Maintenance and Troubleshooting Manual.*

We recommend doing the following if you observe poor reproducibility of AFP values at low levels or you are not satisfied with the performance of the assay.

- Compare the assay reagent and calibrator expiration dates with the dates entered in System Management/Definitions.
- Ensure that you prepared the calibrator, controls, and assay reagents according to the recommended procedures.
- Ensure that you followed the recommended sample collection and handling procedures.
- Remove the septum caps from the reagent vials and check for foam or moisture on the septum caps. Replace the septum caps if necessary.
- Visually check the probe and tubing for obstructions, leaks, and deformities such as pinched or crimped tubing.
- Take further corrective action following procedures established for your laboratory.
- Calibrate the system using new assay reagents, calibrators, and controls.
- Contact the Ciba Corning Technical Assistance Center.

Technical Assistance

For technical assistance, call the Ciba Corning Technical Hotline at 800-255-2121, fax your questions to the Technical Assistance Center at 508-660-4559, or contact your authorized Ciba Corning Distributor.

For customer service, additional information, or to contact your Ciba Corning Account Representative, call 800-255-3232.

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